Persistent Life History Effects of Extended Starvation

by

Moses Antony Sandrof

University Program in Genetics and Genomics
Duke University

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Ryan Baugh, Supervisor

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the University Program in
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ABSTRACT

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Abstract

Starvation during early human development produces epigenetic effects that could be adaptive if famine persists. We modeled the response to early starvation exposure in C. elegans using 'L1 arrest,' a reversible developmental arrest in the first brought on by hatching in the absence of food. We found lifelong developmental effects following recovery from extended L1 arrest. Remarkably, some epigenetic effects persist for multiple generations. After extended starvation, development is delayed, producing smaller adults, fertility is reduced, and stress resistance increases. Starvation causes a striking amount of phenotypic variation among isogenic individuals, and those that develop slowest are least fertile and most stress resistant. However, stress resistance is higher in all L1 arrested animals and dissipates in a growth-dependent manner. We assessed starved animals for signs of autophagy-related feeding defects and found low rates of pumping and feeding as well as grinding defects. A retrospective pumping assay revealed that after extended starvation, animals with lower pumping rates at the L1 stage tended to grow slower. Our work shows that environmental conditions and life history have transgenerational life history effects on several organismal traits, and that these traits appear to be rooted in nutrient-deprivation secondary to autophagy-related feeding defects. However, the manner by which these effects are transmitted transgenerationally remains an open and interesting question.

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Acknowledgements

I would like to thank my lab co-workers, past and present, without whose contributions and support this work would've been impossible.

Introduction

Individual disease-causing genotypes can have a spectrum of effects (Badano and Katsanis, 2002). Similarly, genetically identical individuals respond differently to similar stressors early in life. Whereas some become more resilient, others become more susceptible (Hughes, 2012). The degree of one's stress response arousal in early life has lasting consequences on behavior and life-history traits (Badano and Katsanis, 2002; Casanueva et al., 2011). However, why some individuals respond well and others respond poorly to stress remains largely unclear. The nematode *C. elegans* is an attractive system to study the factors that explain differential stress resistance. Their asexual reproduction allows for production of isogenic populations and separate examination of genetic and epigenetic factors.

Variation in access to nutrients early in life is known to be linked to type-II diabetes as well as other adult metabolic syndromes (Heijmans et al., 2008; Hughes, 2012). Starvation early in development may trigger a whole-organism response that lowers the metabolic set point, possibly in anticipation of further inconsistencies in nutritional supply. This is the "thrifty phenotype" hypothesis thought to explain the increased incidences of heart disease and diabetes in individuals born during the Dutch Famine of 1944 (Hales and Barker, 1992; Schulz, 2010). In recent years, childhood type-II diabetes has increased dramatically in prevalence (Group, 2006). Some studies suggest that this may be partially in part to transgenerational heritability of environmentally-

induced disorders (Badano and Katsanis, 2002). Furthermore, a recent review focusing on early determinants of obesity found interactions between genetic status and epigenetic *in utero* influences (Rhee *et al.* 2012). Understanding the effects of early-life nutrient deprivation both within and between generations has important implications for human health and disease.

Multiple studies have reported transgenerational epigenetic inheritance of simple traits, such as coat color and flower color. However, until recently, transgenerational transmission of more complex traits had not been described. Recently, transmission of heat shock response between generations through incompletely reset chromatin modification was reported. Chromatin modeling-mediated effects on transcription were inherited for up to two generations (Seong et al., 2011). The next year, Brunet's group reported the first instance of epigenetic inheritance of a complex trait. After genetically inducing modifications of parental H3K4 trimethylation, they observed epigenetic inheritance of extended lifespan in the germline (Greer et al., 2011). While these examples do provide evidence of epigenetic transmission of complex traits, they do not provide a context of ecological significance. In the laboratory, this can be achieved by demonstrating analysis of multiple life history traits in the context of a variable ecological landscape.

Phenotypic plasticity can either be adaptive or non-adaptive, depending on other factors in the environment. For example, the "thrifty phenotype" response of increased

anabolism is adaptive in the face of starvation conditions, but adverse in a time of plenty. Likewise, in *C. elegans,* heat shock or starvation may cause organismal sickness and death to part of the population. However, below a certain threshold, these stimuli may induce signaling cascades that could be adaptive if stressful conditions persist (Casanueva et al., 2011; Lee et al., 2012).

Autophagy (literally "self-eating") is a multi-level response of organisms and tissues to environmental or organismal stress (Levine and Klionsky, 2004). It is especially well recognized in the context of starvation, when body tissues or organelles are broken down for energy in the absence of another energy source. For organisms facing an environment with a fluctuating nutrient supply, autophagy provides one important way of maintaining energy homeostasis. Kang et al. (2007) showed that autophagy may act as a pro-survival or a pro-death factor during starvation survival. Mutants constitutively active for autophagy were less resilient to starvation, as were those animals with low levels of autophagy. This dual nature of autophagy suggests a threshold beyond which sustainable levels of autophagy become pathological. The study found morphological and functional disorder of the pharynx in starved animals with hyperactive autophagy (Kang et al., 2007). Animals with slower pumping rates were less likely to fully recover from starvation. Starvation-induced autophagy has an acute detrimental effect on the pharynx, which directly regulates feeding. As the amount of nutrient uptake has been shown to directly regulate key life history traits, it is not

surprising that starvation-induced autophagy produced hormetic effects on life-history traits such as lifespan (Kang and Avery, 2009).

C. elegans is an effective model to study such exposure-disease relationships due to its short generation time and ease of experimental and genetic manipulation. In this and other models, nutrient limitation appears to be linked to a variety of life-history traits, including lifespan extension. When *C. elegans* hatches in the absence of food, it arrests in the first larval stage with increased resistance to stress (Johnson et al., 1984). L1 arrest is characterized by a massive transcriptional shift without morphogenetic change (Baugh et al., 2009). Immediately after recovery on food, starved worms rapidly resume growth and a gene expression profile similar to those seen during normal L1 development. However, our lab has observed that in cases of extended starvation, this rapid resumption of a normal growth program is inhibited, with lingering life history consequences. Additionally, we have seen that some of those consequences are heritable.

We examined the life history effects of extended starvation to ask the question, are there long-term systemic effects of being deprived of food early in life? Our lab has developed a nutrient-deprivation model in *C. elegans* to explore this question. Preliminary unpublished data by Meghan Jobson show that extended starvation conditions early in life have effects in the adult that include increased stress resistance and delayed growth and development. Remarkably, we have observed that some of these phenotypes are heritable. While other studies have demonstrated similar effects

using mutants or RNAi knockdowns, ours is the first to link a heritable complex trait to an exclusively environmental perturbation in a wildtype population.

To follow up on previous results, I asked if our observed phenotypes could be a result of impaired feeding as a result of excessive autophagy of the pharynx, the primary feeding organ. I performed a series of experiments examining the pharyngeal functionality. These experiments demonstrated that feeding ability predicts subsequent life-history traits, suggesting that differential induction of the autophagy response may explain the spectrum of starvation-induced life history effects.

Finally, recent data from our lab has confirmed the presence of a transgenerational effect on developmental delay in the F1 generation. Additionally, the effect was also present in the progeny of heat-shocked individuals, emphasizing the similar stress-like nature of both starvation and heat shock. These data also reveal that all animals passing through L1 arrest acquire increased heat resistance that decreases as a function of the animal's growth. This pivotal finding suggests that the increased heat resistance reported in the population exposed to extended-starvation is a direct consequence of its slow growth.

Methods

Strains

This work used the N2 (wild-type Bristol) strain. Strains were maintained on agar plates supplemented with standard nematode growth media (NGM) seeded with *E. coli* 0P50 at 20°C. N2 animals were maintained continuously to never allow starvation or overcrowding.

Bleaching and L1 arrest

Embryos were prepared by dissolving gravid young adults grown on 10 cm plates in a bleach solution (7:2:1 ddH20, sodium hypochlorite (Sigma), 5 M KOH) for 7-9 minutes total, spinning down the worms and refreshing the bleach solution after 2 minutes. Embryos were washed 3 times in M9 buffer, counted, resuspended at a density of 1 worm per microliter, and cultured in a 25 mm glass test tube on a tissue culture roller drum at 25 rpm at 20-22°C**.** Hatching was scored 16-20 hours post-bleach to confirm health after bleaching. Lethality was scored 1, and 8 d after bleaching to monitor starvation survival. At 8 d survival ranged from 30-80%, and only tubes with 40-60% lethality were plated for recovery and experimental analysis. For recovery, 2,000 arrested L1s were plated per 10 cm plate. Gravid mothers for bleaching were prepared by bleaching and plating 2,000 eggs per 10 cm plate and culturing for 3d.

Image analysis of worm size

At 48 hr recovery, animals were gently washed off plates with M9, washed two times, and plated on room temperature plates with no *E. coli*. 100-300 images were captured per plate at 50x magnification on a Zeiss Discovery V2 stereomicroscope equipped with a Zeiss AxioCam camera. Images were captured using Axiograph software (Zeiss v. 4.8.2) and saved as TIFF files. The Fiji plug-in WormSizer was used to analyze the images producing length and volume estimates (Moore et al., 2013).

Autophagy analysis- grinding

This grinding assay was performed as described by (Kang et al., 2007) with a few modifications. Animals were recovered from starvation on OP50 plates (except for 5hr timepoint). 12 hours before timepoint, animals were washed onto plates seeded with GFP-expressing OP50. Animals were subsequently examined for presence of GFP in the gut and/or mouth under a compound fluorescent microscope.

Autophagy analysis- feeding

Fluorescent beads were added to liquid OP50 culture at a dilution of 1:100 and then seeded onto plates. Animals were recovered from starvation on OP50 plates and washed onto fluorescent bead plates 5hrs before timepoint. Animals were subsequently

scored for number of fluorescent beads in the gut and/or mouth under a compound fluorescent microscope.

Pump rate analysis

Pump rate was determined as described in (Avery and You, 2012). Animals were examined under a tissue microscope at 200X and pharyngeal pumping rate was calculated as the average of two 20-second time periods separated by 5 minutes. In the retrospective study, animals were singled into a 24 well plate before pumping rate was analyzed at the L1 stage. Animal size was later quantified by WormSizer as described above.

Data analysis and statistics

Data were analyzed using Prism (Graph Pad v 5.0) and the R programming language. Statistical tests used are mentioned in the text.

Previous Results

The following are unpublished previous results of a study by Meghan Jobson and Ryan Baugh examining the effects of extended starvation on a variety of life history traits.

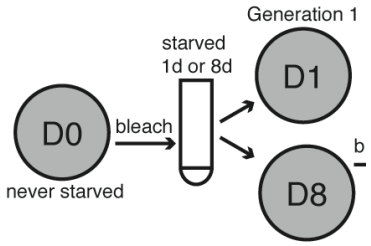

Figure 1: Experimental design. Fed worms were bleached into starvation conditions for either 1 (D1) or 8 (D8) days before synchronous recovery.

In our extended starvation model, animals are starved for either 1 (D1) or 8 days (D8) and then synchronously recovered on food (fig 1). D8 animals are significantly developmentally delayed compared to their D1 counterparts (fig 2A-C). However, given 72 hours recovery, this size discrepancy is attenuated (Fig 2D), suggesting that D8 animals are developmentally delayed rather than smaller overall.

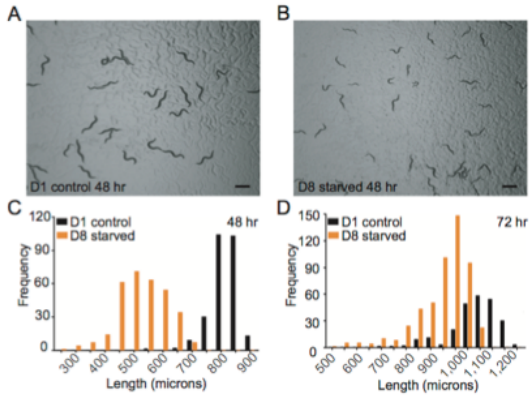

Figure 2: Extended starvation causes developmental delay. D1 worms (A) are visibly bigger than D8 worms (B) at 48 hrs by broad-field microscopy. D1 and D8 worm size was quantified at 48 hrs (C) and 72 hrs (D).

Interestingly, D8 animals are characterized by more variance in growth rate than D1s, indicating a spectrum of delay severity across the isogenic population. Size quantification was conducted by WormSizer, an image analysis program created specifically for this purpose in the Baugh lab (Moore et al., 2013).

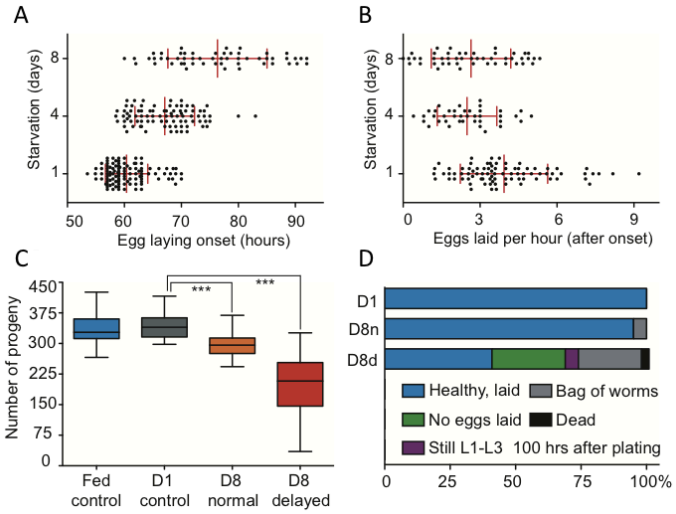

Figure 3: Egg-laying dynamics of animals are altered by extended starvation. Egg laying onset is delayed (A) and slower (B). D8 animals have less progeny overall and D8 "delayed" animals have much fewer progeny. "Delayed" animals also suffer from reproductive abnormalities and sterility at a higher rate than their "normal" counterparts.

Consistent with the finding of developmental delay, D8 animals also show a delayed onset of fertility as well as altered egg-laying dynamics (Fig 3). Onset of egg-laying is delayed about seventeen hours in D8 animals (Fig 3A). Egg-laying rate is also slower in D8 animals.

To determine if the effect is more extensive in the slowest growing animals, after 48 hours recovery we designated D8 animals as either "normal" or "delayed" according to the presence or absence of a vulva, marking entrance into the young adult stage. "Delayed" D8 animals have fewer progeny overall (Fig 3C). They also suffer from reproductive abnormalities such as sterility and premature death by "bag of worms" (Fig 3D). In contrast, "normal" D8s were characterized by egg-laying dynamics

comparable to those of D1s. Similar to the extent of developmental delay, starvation-induced effects on reproduction are also characterized by extensive variability.

The study characterized how stress resistance is affected during recovery from starvation (fig 4). Interestingly, D8 animals are significantly more resistant to heat shock treatment than D1s at 48 hours recovery. Although by this assay all D8 animals appear similarly resistant, we have shown that D8 "delayed" animals are more resistant at more extreme temperatures (data not shown).

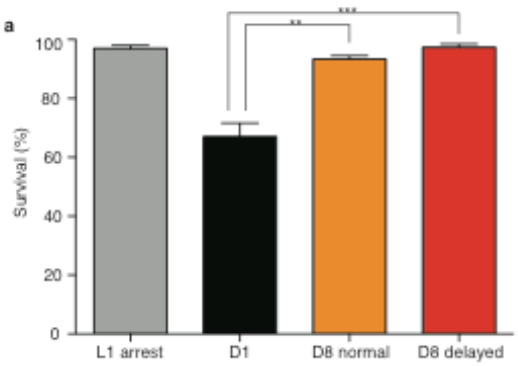

Figure 4: Heat stress is enhanced by exposure to extended starvation.

Our lab has shown that 8 days of starvation has a heritable epigenetic effect on developmental physiology (fig 5). Unstarved progeny of D8 animals (D8D1) are significantly smaller in size after 48 hours growth as progeny of D1 animals (D1D1) (fig 5A). The effect is similar in quality but weaker in intensity than that of D8 animals starved directly. These animals are also more resistant to stress as evidenced by a starvation survival assay (fig 5B). Interestingly, this effect is stronger when the parental

generation is bleached at 96 rather than 72 hours. In accordance with previous data, this effect is stronger in the progeny of "delayed" than "normal" parents (fig 5C).

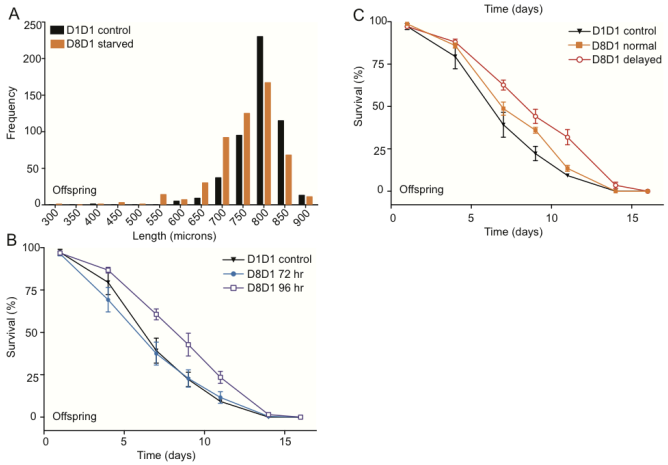

Figure 5: Transgenerational analysis reveals heritability of starvation-induced life history phenotypes. (A) Progeny of starved mothers are slightly developmentally delayed compared to controls. (B) Progeny of starved mothers are also more resistant to L1 starvation, but only when the starved parent population is bleached at 96, rather than 72, hrs. (C) Transgenerational stress resistance is higher in the progeny of the "delayed" than progeny of the "normal" D8 population.

Results

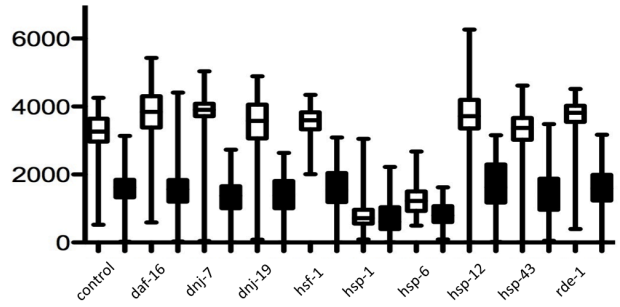

Figure 6: RNAi against selected stress-response genes failed to knock down the delayed-growth phenotype (D1, white; D8, black).

RNAi knockdowns were not able to attenuate the growth delay effect

The most striking extended starvation phenotype is the previous finding of decreased size and growth rate of D8 animals. To test our original hypothesis that developmental delay was a consequence of adaptive signaling in response to environmental stimulus, I recovered D8 animals on RNAi food to knockdown a suite of candidate genes (fig 6). No attenuation of the starvation-induced delay was seen. With hsf-1 knockdown, which delays growth in control populations, D8 animals were still significantly more delayed.

Extended starvation causes aberrant feeding behaviors that are correlated with slow growth

To shed light on the potential pathologic nature of our life history phenotypes, I performed a series of assays to determine the feeding ability of worms subjected to extended starvation. The *C. elegans* esophagus has two main functions: pumping and

grinding. The food is first pumped into the pharyngeal cavity, where it is then ground before being passed into the digestive tract.

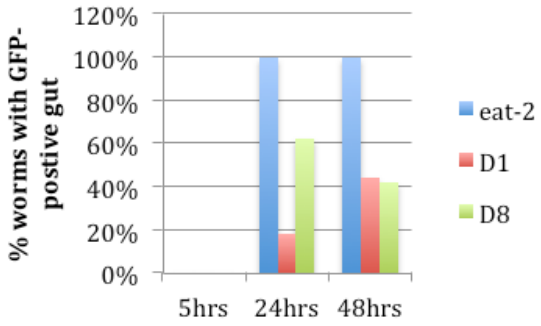

Figure 7: At 24hrs, D8 animals (orange) have more undigested bacteria in their gut than D1 controls (red). Interestingly, at 48hrs, undigested bacteria are present in a significant fraction of both D1 and D8 populations. The *eat*-2 mutant, defective in feeding, was used as a positive control (blue).

Animals were grown on GFP-expressing bacteria. Animals defective for grinding pass the unmasticated bacteria into their gut, whole and fluorescent. Control animals with functional grinders break open the bacteria, exposing the GFP to stomach pH and deactivating it.

At 24 hours recovery, 60% of D8 animals were GFP-positive in the gut compared with less than 20% in the D1 (fig 7). At 5 and 48 hours recovery, treatment did not have an effect on grinding ability. Interestingly, animals tended to accumulate more unground bacteria as they age, independent of treatment. Additionally, no correlation was seen between size at 48hrs and fluorescent bacteria in the gut (data not shown).

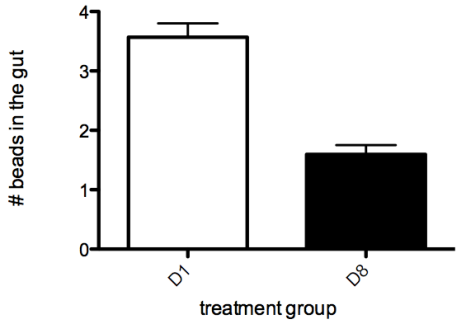

Figure 8: At 24hrs, D8 animals have fewer beads in their gut than D1 controls. Bead counts were quantified in D1 and D8 animals at 24hrs recovery ($p < 0.01$, $n = 100$).

Worms were fed a constant concentration of fluorescent beads for 5 hours and scored for number of beads in the gut. At 24 hours, D8 animals had eaten significantly fewer beads than D1, suggesting a treatment-dependent effect on feeding rate (fig 8).

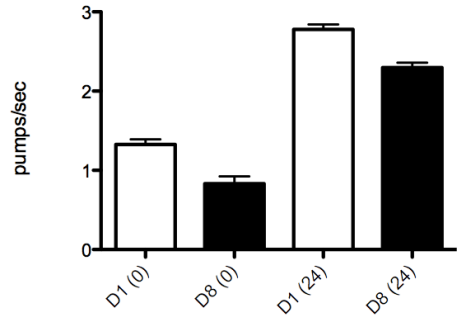

Figure 9: D8 animals have lower pumping rates than D1 controls. Pumping rate was quantified in D1 (white) and D8 (black) animals at 0 and 24 hrs recovery ($p < 0.01$, $n = 50$).

Pumping rate was quantified at 0, 24, and 48 hrs recovery (fig 8). D8 animals had significantly lower pumping rates compared to D1 controls at both 0 and 24 hrs

recovery. At 48 hours, the size difference between D1 and D8 populations precluded a clear comparison of pumping rates.

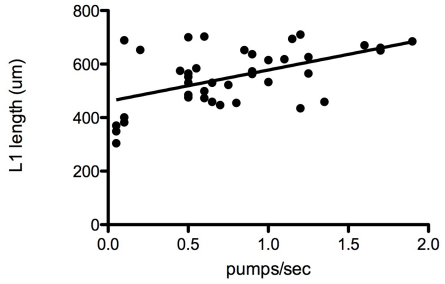

Figure 10: Retrospective assay of D8 pumping rates and size. Pumping rate at the L1 stage was predictive of size at 48hrs ($r^2 = 0.27$, $p < 0.001$).

If the smaller size of D8 animals is a result of decreased feeding caused by slow pumping, it is possible that those D8 animals might have lower pumping rates at the L1 stage. Pumping rates of individual animals were measured at 0hr and length of the same animals was quantified at 48hr. The results are plotted in fig 10. There is a significant correlation between L1 pumping rate and adult size of individual worms ($r^2 = 0.27$, $p < .001$).

Additional Data

The following results were obtained by Jim Jordan to build on the previous transgenerational findings by Meghan Jobson.

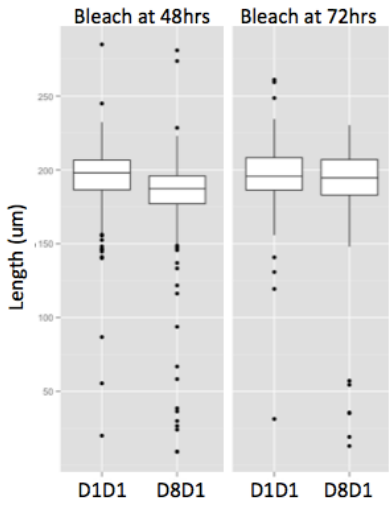

Figure 11: Transgenerational transmission of slow growth. D8D1 animals are smaller than D1D1 at 48hrs recovery. However, the delay phenotype is absent when the parental generation is bleached at 72hrs.

Progeny of D8 mothers who were starved for 1 day (D8D1) were recovered for 48 hours and quantified for size. These animals were smaller in size than control animals whose parents had not been exposed to extended starvation (D1D1) (Fig 11). These results indicate a transgenerational effect of starvation on growth rate. This experiment also compared progeny collected by bleaches at two different timepoints, 72 and 96 hours. Interestingly, only eggs collected at the earlier timepoint gave rise to slow growing individuals.

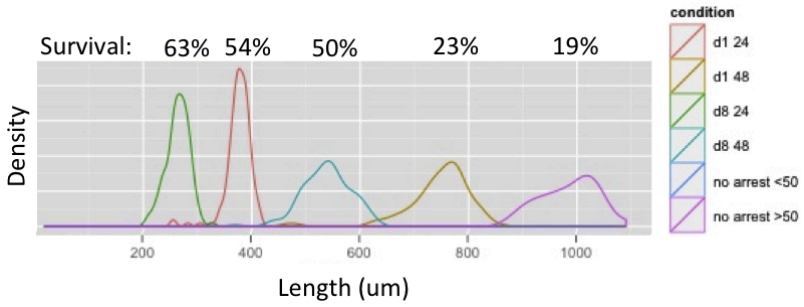

Figure 12: Heat shock resistance in a size-dependent manner independent of treatment. Animals from different treatment groups (D1, D8, never arrested) were recovered on OP50. At 24 or 48 hrs, Size distributions and heat shock survival were determined for whole populations.

Surprisingly, an analysis of heat resistance across various treatments and developmental stages revealed an effect of size on heat resistance dependent only on any time spent in L1 arrest. Heat shock survival assays were performed on D1, D8, and unarrested (D0) animals at 24 and 48 hours of recovery (Fig 11). Juxtaposition of survival rates with size by WormSizer shows that heat shock survival is inversely correlated with size after L1 arrest. Passage through L1 arrest appears to be required for the immediate increase in heat shock resistance.

Discussion

Our results demonstrate that extended starvation at the L1 stage of *C. elegans* leads to significant life history effects throughout development and adulthood, and that some of these changes are heritable. Starvation-induced phenotypes include slow growth, increased stress resistance, and delayed, elongated, and overall decreased fecundity. Additionally, these phenotypes are accompanied by markers of physiologic stress, including an increased incidence of sterility and deaths due to reproductive abnormalities. Animals subjected to extended starvation also exhibit evidence of feeding defects such as slow pharyngeal pumping and insufficient grinding. Strikingly, we have shown that delayed growth and increased stress resistance are heritable traits, but the mechanism of transmission remains unknown. However, evidence from previous studies supports our hypothesis that these traits are passed on by altered maternal provisioning in response to stressful environmental conditions.

Early-life variation of nutrient availability is implicated in later-life manifestation of complex disease states such as type-II diabetes and metabolic syndrome (Heijmans et al., 2008). The increasing prevalence of pediatric type-II diabetes has inspired studies that reveal a potential transgenerational heritability of environmentally-induced disorders. These recent data suggest an epigenetic role in disease progression. Starvation early in development may trigger a whole-organism response that lowers the metabolic set-point, possibly in anticipation of further inconsistencies in nutritional supply (Hales

and Barker, 1992; Schulz, 2010). In addition to early-life cues, evidence suggests that organisms respond to environmental cues received by their parents. While the phenomenological data supporting adaptive epigenetic effects is encouraging, the field remains an active area for study with model organisms.

Due to the ease of isogenic population generation, *C. elegans* has become an appealing model for those studying environmentally-sensitive epigenetic traits. Evidence from our lab has shown that starvation conditions early in life induce strikingly different life history phenotypes from fed controls. Animals hatched in the absence of food arrest in the first larval stage are characterized by dramatically increased stress resistance (Baugh et al., 2009). However, when feeding resumes within the first 24 hours of starvation, these animals rapidly resume normal growth kinetics reflected by a transcriptional program nearly identical to fed controls.

For *C. elegans* in the wild, life is often feast or famine, and nutritional fluctuation is common. We have shown that brief periods of fasting have little to no effects on developmental physiology. However, it was unclear whether this was true following longer periods without food. Our data shows that animals subjected to extended starvation are impeded in their recovery. Previous unpublished data from our lab showed that 8 days of starvation induces striking developmental delay, increased stress resistance, and altered egg-laying dynamics (figs 2, 3, 4). In addition, extensively starved isogenic populations display significantly increased variance in growth rate compared to

unstarved controls (fig 2). These data suggest a sophisticated mechanism regulating developmental physiology in response to environmental conditions. However, it is unclear whether these mechanisms represent distinct developmental pathways or a spectrum of buffered responses to stress.

I tested two hypotheses to clarify the connection between early starvation exposure and later life physiology. The first, an adaptive hypothesis, involves starvation inducing a signaling cascade that leads to activation of a second "resilient" developmental program. In the second, starvation-induced pathology overwhelms the system, compromising normal physiological function. While these two hypotheses are not mutually exclusive, they represent two extreme possibilities of how organisms respond to stress. In other words, they might describe the "resilient" and "sensitive" ends of the stress-response spectrum (Hughes, 2012).

Altered life history traits are not the result of stress-response signaling activation

The first hypothesis, which involves activation of a distinct life history program, implies that slow growth in the starved population is a developmental decision not rate-limited by caloric intake. If each animal were physically capable of growing at the normal rate, knockdown of the delayed phenotype might be achieved using RNAi or a mutant strain. A strong candidate for one such regulator might be one of the many heat shock chaperone proteins involved the unfolded-protein response. It is accepted in the literature that the UPR is activated by starvation conditions in many organisms.

Additionally, activation of the unfolded protein response specifically in the gut is capable of arousing a whole-body stress response (Durieux et al., 2011). A recent study in *C. elegans* showed that variation in a heat-shock response early in life could predict mutation penetrance later in life (Casanueva et al., 2011). Similarly, variance in the degree of starvation-induced developmental delay could be explained by variability in starvation-induced stress-response.

Animals exposed to extended starvation were recovered on bacteria expressing RNAi of genes involved in the heat shock response and UPR pathways. However, the severity of D8 growth delay compared to control was not affected for any genes tested (fig 6). Additionally, I examined a suite of heat shock response GFP-reporter strains exposed to extended starvation. However, I found no changes relative to control (data not shown). Together, these data suggest that an unfolded protein response-mediated pathway is not mechanistically responsible for the observed life history phenotypes.

Autophagy-induced pathology causes developmental delay

Other studies have shown evidence that animals subjected to extended starvation are more likely to suffer from reproductive and feeding abnormalities (Harvey and Orbidans, 2011; Kang et al., 2007). However, growth rate was not addressed in these studies. Our lab has shown by assaying starvation-resistance at the L3 stage that D8 animals are more sensitive to starvation-induced stress (data not shown). If autophagy-mediated degradation of the pharynx is significant, D8 animals might only be capable of

an extremely limited growth rate. Additionally, starvation-induced autophagy is not limited to the pharynx, but systemic tissues as well.

To test for feeding-related pathology, I conducted a series of experiments to assay D8 animals for signs of autophagy-mediated pathology (figs 7-10). Results from these experiments suggest that D8 animals have trouble properly grinding and pumping, and that they pump slower and eat less. Finally, a retrospective analysis of pumping rate shows that the smaller animals at 48 hours tended to pump slower as early larvae (fig 10). Taken together, these results support the hypothesis that D8 animals grow more slowly because of limited feeding ability.

Developmental delay underlies other life history phenotypes

Recent experiments in our lab have untwined the delayed growth and stress resistance phenotypes, revealing that both D1 and D8 animals are initially stress resistant after L1 arrest but lose this trait as a function of growth (fig 12). Previous experiments failed to correct for the D1 animals' larger size. This new finding indicates that heat resistance in the D8 is a secondary phenotype to developmental delay.

Tissue damage explains inconsistencies in starvation-induced lifespan

Caloric restriction inhibits reproduction (Guarente, 2005; Partridge and Harvey, 1988). *C. elegans* habitat tends to be food or famine, so it is possible that this is an adaptive measure to delay reproduction during times of low food supply. Food signals and reproductive cues are integrated in the worm to make developmental decisions

about life history traits such as aging (Crawford et al., 2007). Reproductive status directly affects aging, as removal of the germ cells extends lifespan (Hsin and Kenyon, 1999). However, removal of the somatic reproductive tissues as well as the germline does not extend lifespan. Our model did not reveal conclusive evidence for extended lifespan. Previous studies have revealed that extended-starvation causes extensive damage to the gonad, possibly interfering with this signaling pathway (Lee et al., 2012). I speculate that systemic tissue damage to the gonad and other systems may be the reason why extended lifespan is not reproducibly observed in our extended-starvation model.

Transgenerational effects are likely the result of aberrant maternal provisioning

We have seen heritability of two complex traits, growth rate and stress resistance. Unstarved progeny of D8 mothers grow more slowly (figs 5A, 11, 12). We initially reported that these animals were more stress resistant (figs 5B, 5C), but it is likely that this is dependent on the slow growth phenotype.

Our data suggest a transmission mechanism directing the passage of characteristics affecting a variety of life-history traits from parents to offspring. Recent work has offered a number of potential candidates for transmission. ATF-2 was found to be required to transmit a stress-induced epigenetic change by altering transcriptional dynamics by inhibiting euchromatin formation(Seong et al., 2011). Another group found that parental deficiencies in H3K4me3 modifiers extended lifespan in descendants

(Greer et al., 2011). Finally, a recently published study in Nature showed that multigenerational inheritance of RNAi-mediated gene silencing was dependent on the nuclear Argonaute protein HRDE-1 (Buckley et al., 2012).

Another study revealed effects of caloric-restriction on maternal loading of nutrients into the egg (Harvey and Orbidans, 2011). This study found that calorically-restricted mothers produced progeny well adapted to poor nutritional environments. However, these same animals suffered compared to controls in good nutritional environments. This study is reminiscent of our work with this experimental group, which show decreased growth on normal conditions but increased hardiness against starvation-induced stress. However, our results indicate that any hardiness against starvation in the second generation is a result of passing through L1 arrest rather than a transgenerational effect. As discussed above, stress resistance could be subtly responsive to a transmitted effect on growth rate as well.

Recent research suggests that extended starvation causes irreparable damage to the gonad (Lee et al., 2012). Our results show decreased fecundity and increased incidence of infertility and reproductive defects, findings that support this conclusion. Because this effect occurs on a spectrum, it is likely that some surviving eggs in the parental generation were not properly loaded with sufficient nutritional content for proper development. This theory is supported by observations in our lab that extended-starved L1s have less fat than control L1s by Oil Red O staining (data not shown). These data

support the hypothesis that starvation-induced heritability is likely the result of gonad pathology as opposed to adaptive signaling of an environmental response.

However, if an heritable signaling response to low nutrient conditions does exist, there is the possibility that it has been overwhelmed by the severe response caused by 8 days of starvation. Further research is needed to solidify the pathway connecting the pathology accompanying extended starvation from the life history effects. A dose response experiment examining subacute levels of starvation could reveal subtle life history modulations or phenotypes that are potentially lost with excessive pathology.

Recent data from our lab has shown that heat shock can act as a proxy for our extended starvation treatment. Heat shock treatment in the L1 has similar effects on growth rate and accompanying life history characteristics, making it a good candidate treatment for future experiments. Additionally, it is more efficient and conducive to RNAi screens than starvation.

The experiments described in this thesis reveal a striking effect of extended starvation on growth rate. It appears that this phenotype is linked to autophagy-induced pathology of the feeding organ that prevents sufficient nutrient uptake to support a normal growth rate. Animals exposed to extended starvation also appear to be more stress resistant. However, stress resistance is granted by even one day spent in L1 arrest and decreases in a growth-rate-dependent manner. Changes in reproductive dynamics likely result from both slow growth as well as starvation-induced damage to the gonad.

It is unclear yet how these phenotypes are transmitted to offspring, but germ cell pathology is likely involved.

This study sets the stage for exciting future research furthering our knowledge of environmentally-responsive transgenerational biology with exciting implications for the interface between ecology and organismal physiology.

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